American Journal of Hematology 52:305–309 (1996)

Hemoglobin S/Hemoglobin Osler: A Case With 3 β Globin Chains. DNA Sequence (AAT) Proves That Hb Osler Is β 145 Tyr→Asn

P. Jesse Hutt, Milton H. Donaldson, Jamil Khatri, Virgil F. Fairbanks, James D. Hoyer, Stephen N. Thibodeau, Michael S. Moxness, Lydia E. McMorrow, Michael M. Green, and Richard T. Jones

Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, Minnesota (P.J.H., V.F.F., J.D.H., S.N.T., M.S.M., M.M.G); Cooper Hospital/University Medical Center, Camden, New Jersey (M.H.D., J.K., L.E.M.); Department of Biochemistry and Molecular Biology, University of Oregon, Portland (R.T.J.)

A 13-year-old African-American female with erythrocytosis and three different β globins on electrophoresis β^A, β^S, and β^{Osler}, raised the possibility that one chromosome 11 might contain a duplicated β globin gene, since there are normally only 2 β globin genes. DNA sequence analysis showed GTG at codon 6 in exon 1, corresponding to Hb S and AAT at codon 145 in exon 3, indicating a substitution of Asn for Tyr. Thus, Hb Osler undergoes spontaneous post-translational deamidation, β 145 Asn→β 145 Asp. Unmodified Hb Osler (Asn) co-migrates with Hb A on electrophoresis and co-elutes with Hb A on HPLC; therefore it has not been identified previously. All previous studies have incorrectly identified the mutation as being β 145 (HC 2) Tyr→Asp.

Key words: abnormal hemoglobins, polycythemia, Osler, PCR, DNA, deamidation

INTRODUCTION

Hemoglobin (Hb) Osler is a high oxygen affinity hemoglobin associated with erythrocytosis [2–4]. It was reported as β 145 (HC 2) tyrosine→aspartic acid. Hb Fort Gordon and Hb Nancy were also reported as β 145 Asp. However, DNA analysis proves a different mutation in the grandchild and in the original case of Hb Osler.

CASE REPORT

The patient is a 13-year-old African-American female, the granddaughter of the index case originally reported as having Hb Osler [2]. She complained of headaches and tingling of her face, arms, and hands lasting 1 to 2 days at a time. She has a non-identical twin sister. Her physical examination was remarkable only for plethora and warm palms. She had a venous hemoglobin concentration level of 19.7 g/dL, RBC count of 7.65×10^{12}, MCV of 78 fL, and hematocrit 0.60. The white cell and platelet counts were normal. Alkaline electrophoresis at pH 8.6 showed a "fast" variant migrating in the Hb J position (39.6%). There were two additional bands, at the Hb S and Hb A positions (32.7% S, 24% "A", 2.9% A2, and 0.8% F). Acid citrate agar electrophoresis (pH 6.2) showed bands at the Hb S and Hb A positions, and a faint band at the Hb F position (see Figs. 1 and 2). Chromatography using a Variant HPLC column (Bio-Rad Diagnostics, Hercules, CA) confirmed three major Hb peaks. Globin chain electrophoresis in 8M urea at both alkaline and acid pH showed three distinct β globins in the positions of β^J, β^A, and β^S. The presence of three different globin chains was confirmed by reversed phase HPLC separation using a C-4 column. Hemoglobin stability tests were done with both isopropanol and heat and

Received for publication December 12, 1995; accepted March 6, 1996.

Address reprint requests to Virgil F. Fairbanks, M.D., Mayo Clinic, 200 First Street S.W., Rochester, MN 55905.

Results of this study were presented at the 37th Annual Meeting of the American Society of Hematology, December 4, 1995.

Fig. 1. Alkaline electrophoresis, pH 8.6, cellulose acetate. Top: A control from a mixture of Hb S trait blood with Hb C trait blood. Bottom: The patient, showing 3 hemoglobin bands; in order from the anode, these are Hb Osler (Asp), Hb Osler (Asn) and Hb S.

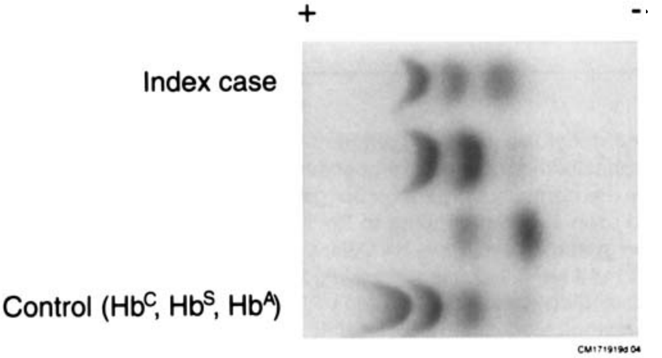

Fig. 2. Acid citrate agar electrophoresis (pH 6.2). From top to bottom, the specimens are from the patient, from a case of Hb S trait, from a neonate, and from a (control) mixture of Hb S trait blood and Hb C trait blood.

were normal. The test for solubility of deoxy Hb S was positive.

Possible explanations for the three β globin chains are: (1) the patient is a chimera with two different clones of erythroid precursor cells; (2) there are two β globin genes on one chromosome 11; (3) a single point mutation results in two different β globins.

In order to test these possibilities, cytogenetic studies and DNA sequencing of the β globin gene were performed. The results of DNA sequence analysis led to a repeated examination of Hb Osler by amino acid sequence analysis.

MATERIALS AND METHODS

Blood specimens from the patient were collected with K_3EDTA as an anti-coagulant. Cytogenetics studies were performed on phytohemagglutin-stimulated lymphocytes by standard procedures and analyzed by GTW and Q-banding methods [5].

The three exons of the β globin gene were amplified by polymerase chain reaction, using the primers shown in Table I. The final volume contained 250 ng DNA, 50 mM KCl, 10 mM Tris-Hcl, pH 8.3, 2.0 mM $MgCl_2$, 200 μM each deoxyribonucleotide triphosphate (dATP, dCTP, dTTP, dGTP), and 1.2 U of *Taq* polymerase. The amplification was performed at 94°C for 30 sec, 60°C for 30 sec, and 72°C for 30 sec for a total of 30 cycles. After the last cycle, the samples were incubated for an additional 10 min at 72°C. The presence of amplified template was confirmed using a 3% agarose gel with ethidium bromide.

DNA was sequenced using the fmol sequencing kit (Promega, Madison, WI). A 1/10 dilution of the product was used for each sequencing reaction. The sequencing primers are listed in Table I, as are the optimal annealing temperatures for the three different exons.

Globin chain separation was performed by chromatography of the hemolysate with a C4 large-pore reverse-phase high-performance liquid chromatography column [6]. The separated globin chains were digested with trypsin, and a chromatogram of the resultant peptides was obtained by reverse-phase high-performance liquid chromatography with a C18 column [7]. The abnormal peptides were analyzed for their amino acid compositions. The last two residues of the C-terminal sequences of the globin chains were determined by hydrolysis with carboxypeptidase P in 0.05 M ammonium acetate at pH 3.7 and sampling at 1, 2, 4, and 8 min followed by amino acid analysis without HCl hydrolysis [8].

RESULTS

Chromosome analysis of blood lymphocytes by Q-banding revealed normal female karyotypes in both the patient and the twin sister. There was no evidence of chimerism.

DNA nucleotide sequence (Fig. 3) confirmed GTG at codon 6 of exon 1, the mutation of Hb S. In exon 2, no mutations were detected. Exon 3 (Fig. 4) contained a mutation at codon 145 (TAT→AAT) which corresponds to Tyr→Asn.

These results prompted re-examination of the amino acid sequence of the affected β globin chains, which confirmed that Hb Osler is β145 Asn.

A specimen of blood was subsequently received from

TABLE I. Primers for Amplification and for Sequencing*

Exon			Length (bp)	Annealing temperature
Amplification primers (5′→3′)				
1	F	GTACGGCTGTCATCACTTAGAC	22	
	R	AAACCCAAGAGTCTTCTCTGTC	22	
2	F	ACTGGGCATGTGGAGACAGAGA	22	
	R	TGTTTCCCATTCTAAACTGTAC	22	
3	F	CATATTGCTAATAGCAGCTACA	22	
	R	ATTTTCCCAAGGTTTGAACTAG	22	
Sequencing primers (5′→3′)				
1	F	ACACCCTAGGGTTGGCCAATCTACTC	26	70°
	R	ATGCCCAGTTTCTATTGGTCTCCTTAA	27	
2	F	TTGGGTTTCTGATAGGCACTGACTCT	26	65°
	R	TTCTCCCCTTCCTATGACATGAACTTA	27	
3	F	TAAGGCTGGATTATTCTGAGTC	22	70°
	R	GACTTAGGGAACAAAGGAACCTTTAATA	28	

*F, forward; R, reverse.

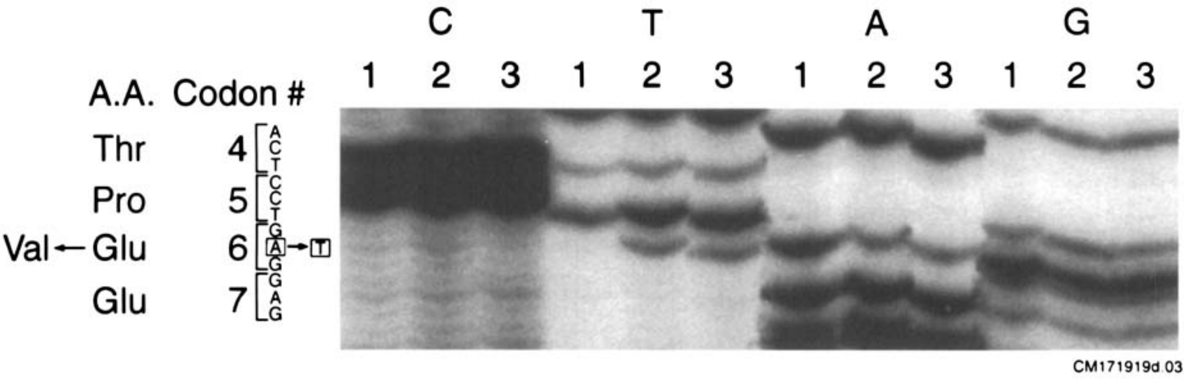

Fig. 3. The Hb S mutation (GAG→GTG) in exon 1 of the β globin gene. Lanes are labeled with the dideoxynucleotide. Lane 1 is a normal control; lane 2 is a known Hb S trait; lane 3 is the patient.

the patient's grandmother, who was the original Hb Osler index case. (Because of the difference in surnames, it was not recognized in the laboratory as being from a relative until after DNA sequencing was completed, and it was not initially identified as Hb Osler). Alkaline electrophoresis of this specimen showed a band anodic to Hb A, matching with Hb Osler (Asp), and comprising 35% of the total hemoglobin. Her β-globin gene was also subjected to DNA sequence analysis, which confirmed the mutation TAT→AAT at codon 145.

DISCUSSION

This patient, who demonstrated three different β globin genes on electrophoresis and chromatography is a compound heterozygote for Hb S/Hb Osler. DNA sequence analysis demonstrated that Hb Osler is β 145 Tyr→Asn rather than β 145 Tyr→Asp as previously reported. However, in addition to Hb S, the patient has two abnormal bands on electrophoresis. The anodic band is the deamidated form β145 Asp, which should be designated Hb Osler (Asp), and the Hb band appearing to be Hb A is the unmodified Hb Osler, or Hb Osler (Asn). Since prior studies of amino acid sequence of Hb Fort Gordon and Hb Nancy also considered only the electrophoretically fast anodic band (β145 Asp), those cases should be reexamined to determine whether they are also due to deamidation of β145 Asn, or whether they really are β 145Asp.

While these studies were in progress, Kattamis et al. (personal communication) independently studied another case of Hb Osler by DNA sequencing, and have confirmed the mutation TAT→AAT in β gene codon 145. Their case is also in an African-American child of the Philadelphia, PA/Camden, NJ community, and may be of the same kindred as the cases here reported.

Post-translational deamidation has also been described in Hb Providence [9,10] [β 82(EF6)Lys→Asn and Asp], Hb Wayne [11] [α139–141 extended], and Hb Redondo

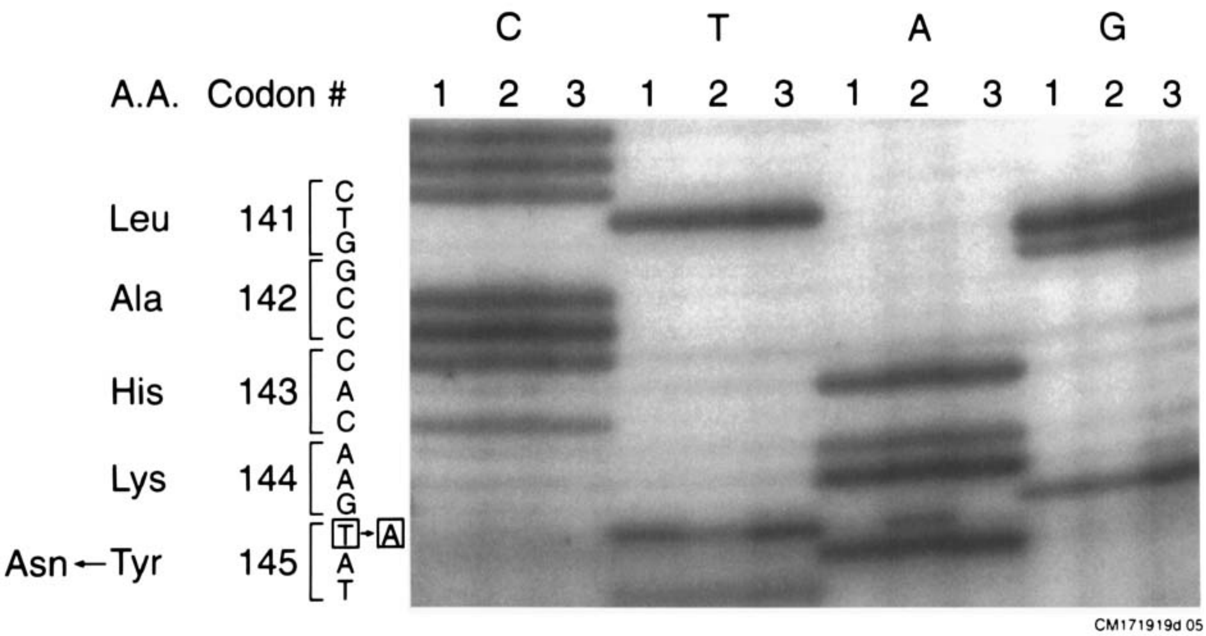

Fig. 4. The Hb Osler mutation (TAT→AAT) in exon 3 of the β globin gene. Lanes are labeled with the dideoxynucleotide. Lanes 1 and 3 show normal sequence; lane 2 is the patient.

[12] [β92(F8)His→Asn], each causing the electrophoretic finding of two anomalous hemoglobin bands. It has been reported that an adjacent histidine promotes deamidation of asparagine [13–17]. Furthermore, studies with 34 synthetic peptides using the sequence Gly-X-Asn-Y-Gly showed that deamidation is especially accelerated when the peptide has histidine in position Y, i.e., the carboxyl of asparagine binds to the α-amino group of histidine [18]. Thus, in Hb Osler, the normal β146 His would promote deamidation of Hb Osler (Asn) to Hb Osler (Asp). Similarly, in Hb Redondo, the mutation places asparagine adjacent to histidine. However, this relationship to histidine cannot be invoked for Hb Providence or Hb Wayne, for in neither of these variants is histidine in proximity to asparagine in the primary structure of the mutant globin chain.

Spontaneous post-translational deamidation has also been reported for hemoglobin variants La Roche-sur-Yon [19] [β81(EF4)Leu→His] and J-Singapore [20] [α79 (EF)Ala→Gly], in which abnormal folding of the EF corner brings a histidine into such a position that it catalyzes the deamidation of asparagine at a site other than that of the amino acid substitution. A similar mechanism of folding that juxtaposes histidine and asparagine may be responsible for post-translational deamidation of hemoglobins Providence and Wayne.

Except for unstable Hb variants, those β chain Hb variants that are anodic to Hb A on alkaline electrophoresis typically exceed the proportion of Hb A in a ratio of about 55:45. Reports that Hb Osler was present as 30% of total hemoglobin have, therefore, been puzzling. This riddle has now been resolved by the demonstration that unmodified Hb Osler co-migrates with Hb A, and that Hb Osler (Asn) plus Hb Osler (Asp) together comprise about 60% of the total hemoglobin.

CONCLUSION

Hb Osler results from the mutation TAT→AAT in codon 145 of the β globin gene. This is translated to β 145 Tyr→Asn, which spontaneously deamidates to β 145 Asp, thus giving rise to two Hb variants, unmodified Hb Osler (Asn) and deamidated Hb Osler (Asp). The former co-migrates with Hb A on electrophoresis; the latter is anodic to Hb A. Previous studies have characterized only Hb Osler (Asp).

ACKNOWLEDGMENT

The authors gratefully acknowledge the excellent technical assistance of Ms. Kathy Kubik, Ms. Charlotte Head, and Ms. Regina Scheidt. This study was supported in part by NIH grant no. HL20142.

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